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Technical Datasheet

RPMI-1640 (Modified for Autoclaving)

Without L-Glutamine and Sodium bicarbonate

Product Code: AT126A

Product Description :

Roswell Park Memorial Institute (RPMI) media are a series of media developed by Moore et al for the culture of human normal and neoplastic cells in vitro. RPMI-1640 is the most commonly used medium in the series. A modification of McCoy's 5A medium, the medium was specifically designed to support the growth of human lymphoblastoid cells in suspension culture. Presently the medium is extensively used for a wide range of anchorage dependant cell lines. The medium needs to be supplemented with 5-20% fetal bovine serum. The medium is also known to support growth of cells in the absence of serum.

AT126A is RPMI-1640 and is modified for autoclaving. It does not contain L-glutamine. Autoclavable media offer a convenient alternative to membrane sterilized liquid medium. It is modified to include heat stable components to ensure that product efficacy is maintained after autoclaving. L-glutamine is heat labile, hence has been omitted from the formulation. Users are advised to review the literature for recommendations regarding medium supplementation and physiological growth requirements specific for different cell lines.

Composition :

Ingredients	mg/L
INORGANIC SALTS	
Calcium nitrate tetrahydrate	100.000
Disodium hydrogen phosphate	800.000
Magnesium sulphate anhydrous	48.840
Potassium chloride	400.000
Sodium chloride	6000.000
AMINO ACIDS	
Glycine	10.000
L-Arginine hydrochloride	241.870
L-Asparagine monohydrate	50.000
L-Aspartic acid	20.000
L-Cystine dihydrochloride	65.200
L-Glutamic acid	20.000
L-Histidine hydrochloride monohydrate	20.270
L-Hydroxyproline	20.000
L-Isoleucine	50.000
L-Leucine	50.000
L-Lysine hydrochloride	40.000
L-Methionine	15.000
L-Phenylalanine	15.000
L-Proline	20.000
L-Serine	30.000
L-Threonine	20.000
L-Tryptophan	5.000
L-Tyrosine disodium salt dihydrate	28.830
L-Valine	20.000
VITAMINS	
Choline bitartarate	5.440
D-Biotin	0.200
D-Ca-Pantothenate	0.250
Folic acid	1.000
Niacinamide	1.000
Pyridoxine hydrochloride	1.000
Riboflavin	0.200
Succinic acid (free acid)	75.000
Succinic acid hexahydrate sodium salt	100.000
Thiamine hydrochloride	1.000
Vitamin B12	0.005
i-Inositol	35.000
p-Amino benzoic acid (PABA)	1.000
OTHERS	
D-Glucose	2000.000
Glutathione reduced	1.000
Phenol red sodium salt	3.180

Directions :

1. Suspend 10.3gms in 900ml tissue culture grade water with constant, gentle stirring until the medium is completely dissolved. Do not heat the water.
2. Adjust the pH to 4.0 before autoclaving.
3. Make up the volume to 963ml (This volume is derived after subtracting the volume of 7.5% sodium bicarbonate solution and 200mM L-glutamine solution from the final volume).
4. Dispense in separate containers or bottles.

5. Autoclave medium at 121°C at 15psi for 15minutes.
6. Remove the medium promptly from the autoclave to avoid extended heating or evaporation.
7. Allow to cool at room temperature.
8. Add 26.7ml of 7.5% sodium bicarbonate solution (TCL013) and 10.3ml of 200mM L-glutamine solution (TCL012) to the final volume of the medium being prepared.
9. If necessary, adjust the pH using sterile 1N NaOH (TCL002) or 1N HCl (TCL003).
10. Store liquid medium at 2-8°C and in dark till use.

Material required but not provided :

Tissue culture grade water (TCL010)
Sodium bicarbonate solution 7.5% (TCL013)
1N Hydrochloric acid (TCL003)
1N Sodium hydroxide (TCL002)
L-Glutamine solution 200mM (TCL012)
Foetal bovine serum (RM1112/RM10432)

Quality Control:

Appearance
White to light pink, homogenous powder

Solubility
Clear solution at 10.3 gms/L.

pH without Sodium Bicarbonate
6.70 -7.30

pH with Sodium Bicarbonate
6.60 -7.20

Osmolality without Sodium Bicarbonate (mOsm/Kg H_2O)
220.00 -260.00

Osmolality with Sodium Bicarbonate (mOsm/Kg H_2O)
285.00 -315.00

Cultural Response
The growth promotion capacity of the medium is assessed qualitatively by analyzing the cells for the morphology and quantitatively by estimating the cell counts.

Endotoxin Content
NMT 1EU/ml

Storage and Shelf Life:

1. All the powdered media and prepared liquid culture media should be stored at 2-8°C. Use before the expiry date. Inspite of above recommended storage condition, certain powdered medium may show some signs of deterioration /degradation in certain instances. This can be indicated by change in colour, change in appearance and presence of particulate matter and haziness after dissolution.
2. Preparation of concentrated medium is not recommended since free base amino acids and salt complexes having low solubility may precipitate in concentrated medium.
3. pH and sodium bicarbonate concentration of the prepared medium are critical factors affecting cell growth. This is also influenced by amount of medium and volume of culture vessel used (surface to volume ratio). For example, in large bottles, such as Roux bottles pH tends to rise perceptibly as significant volume of carbon dioxide is released. Therefore, optimal conditions of pH, sodium bicarbonate concentration, surface to volume ratio must be determined for each cell type. We recommend stringent monitoring of pH. If needed, pH can be adjusted by using sterile 1N HCl or 1N NaOH or by bubbling in carbon dioxide.
4. If required, supplements can be added to the medium prior to or filter sterilization observing sterility precautions. Shelf life of the medium will depend on the nature of supplement added to the medium.